REMARKS

I. Amendments to the Claims

Claims 4-10, 16-18, 20, 21, 26, 29, 31-33, 36-39, 42, and 44-54 have been amended in order to more distinctly point out and claim that which Applicants regard as their invention and to be consistent with the issued claims of the parent application. The amendments to the claims are purely formal with the sole exception of the amendment to Claim 50. Support for the amendments to the claims is found throughout the specification and claims as originally filed. Support for the amendment to Claim 50 may be found, for example, in the specification at page 28, lines 12-19. No new matter has been added by way of these amendments. A marked-up copy of the amended claims is attached hereto as Appendix A, while a copy of the claims pending in the instant application is attached hereto as Appendix B. Claims 1-54 are currently pending.

II. Enablement of the Claims under 35 U.S.C. § 112

Claims similar to those pending in the instant application were rejected under to 35 U.S.C. § 112 as allegedly not enabled by the specification during prosecution of the parent application. Applicants respectfully submit that currently pending Claims 1-54 are fully enabled and thus meet the statutory requirements for patentability under 35 U.S.C. § 112.

Under 35 U.S.C. § 112, a specification must describe a claimed invention sufficiently to enable one of ordinary skill in the art to practice the invention without undue experimentation. A multi-factor test summarized by the Federal Circuit in *In re Wands* forms the basis for an inquiry into whether an amount of experimentation is undue. These factors include (1) the quantity of experimentation necessary, (2) the amount of guidance provided, (3) the presence or absence of working examples, (4) the nature of the invention, (5), the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. *See In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (1988). The test for determining whether experimentation is undue is "not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine or the specification provides a reasonable amount of guidance with respect to ... the experimentation." *See Ex parte Jackson*, 217 U.S.P.Q. 804, 807 (1982).

Applicants respectfully submit that Claims 1-54 are fully enabled by the specification. Claims 1-54 recite, among other elements, a recombinant thermostable DNA polymerase which is characterized in that in its native form, the polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4, 6, 9, and 10 of said sequence are any amino acid residue, and "Xaa" at position 7 of said sequence is Val or Ile; the "Xaa" at position 4 is mutated in comparison to the native sequence, except that "Xaa" at position 4 is not mutated to Glu; and the thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of the polymerase. The specification as originally filed fully enables one of skill in the art to make and use such a recombinant thermostable DNA polymerase.

The Declaration of David Gelfand filed herewith provides evidence of the enablement of the claims, showing that no undue experimentation is required to practice Claims 1-54. The declaration shows that the specification teaches one of ordinary skill in the art to make and use the recombinant thermostable DNA polymerases of the invention without undue experimentation and that the claimed recombinant thermostable DNA polymerases exhibit reduced discrimination against fluorescein family dye-labeled nucleotides.

For example, the Declaration shows that the detailed instructions provided by the specification in the working examples allow one of skill in the art to make and use a recombinant thermostable DNA polymerase with reduced discrimination against fluorescein family dye-labeled nucleotides as recited by the claims, without undue experimentation. Following the teaching of the specification, all 19 possible mutants substituted at the position four of the critical motif of a recombinant thermostable DNA polymerase were constructed with only routine experimentation. *See* the Declaration of David Gelfand, paragraph 8, and the specification at page 17, line 25 to page 18, line 8. All of these mutants have reduced discrimination against nucleotides labeled with fluorescein family dyes, as verified by routine experiments based on the teaching of the specification. *See* the Declaration of David Gelfand, paragraphs 10-17, and the specification at page 35, line 22 to page 38, line 3. Thus, the specification provides ample guidance in its working examples to practice the invention as currently claimed without undue experimentation.

In addition, the Declaration demonstrates that one of only ordinary skill in the art can make and use the recombinant thermostable DNA polymerases which are mutated at position four of the critical motif to an amino acid other than glutamate without undue experimentation, as the average level of skill of those in the art and the state of the art are high. Specifically, the relevant arts, including molecular biology, biochemistry, and enzymology, are well-developed, with a large number of commonly known methods that can be used to construct and characterize the recombinant thermostable DNA polymerases as taught by the specification. For example, the site-directed mutagenesis methods, competition assays, and direct incorporation extension assays described in the declaration are all well known in the art and were easily performed by one of ordinary skill. Accordingly, the declaration establishes that one of skill in the art could make and use the polymerases of the invention without undue experimentation.

Finally, the declaration shows that the specification enables the full scope of the claims. The experimental results demonstrate that each recombinant thermostable DNA polymerase that contains a critical motif wherein the amino acid at position four is mutated to a residue other than glutamate exhibits significantly reduced discrimination against nucleotides labeled with fluorescein family dyes. *See* the Declaration of David Gelfand, paragraphs 10-13 and 16-17. Thus, each recombinant thermostable DNA polymerase recited by the claims of the instant application can be used to practice the disclosed methods. Therefore, the specification is fully enabling with respect to the entire scope of the invention as presently claimed.

Accordingly, Applicants respectfully submit that the guidance provided by the specification, as evidenced by the Declaration of David Gelfand, allows one of ordinary skill in the art to make and use the claimed invention without undue experimentation. Thus, the specification fully enables the invention as presently claimed as required under 35 U.S.C. § 112, first paragraph.

III. Written Description of the Claimed Subject Matter under 35 U.S.C. § 112

During prosecution of the parent application, claims similar to those pending in the instant application were also rejected pursuant to 35 U.S.C. § 112 as allegedly not sufficiently described by the specification to reasonably convey to one skilled in the art that Applicants

possessed the claimed invention. Applicants respectfully submit that the subject matter of Claims 1-54 is sufficiently described by the specification and thus meets the statutory requirements for patentability under 35 U.S.C. § 112.

In particular, the PTO asserted that the specification does not teach how the F667Y and G46D mutations would function in reducing the discrimination of incorporation of fluorescein family dyes. The PTO also alleged that the specification does not describe a representative number of species of recombinant thermostable DNA polymerases encompassed by Claims 1-54.

According to the legal standard for the written description requirement of 35 U.S.C. § 112, a specification must "convey with reasonable clarity to those skilled in the art that, as of the filing date sought, [the applicant] was in possession *of the invention*. The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed*." *See Vas-Cath Inc. v. Mahurkar*, 19 U.S.P.Q.2d 1111 (Fed. Cir. 1991) (emphasis in original).

As an initial matter, Applicants respectfully submit that the currently pending claims are fully supported by the specification and claims as originally filed.

Regarding the PTO's first objection, Applicants respectfully submit that the F667Y and G46D mutations do not affect discrimination against incorporation of fluorescein family dye-labeled nucleotides. The PTO's attention is respectfully invited to the specification at page 35, lines 22-24; page 37, lines 20-30; and page 38, lines 1-4. Here, the specification describes an experiment comparing discrimination against fluorescein family dye-labeled nucleotides by the G46D F667Y double mutant DNA polymerase to discrimination by the G46D F667Y E681K triple mutant DNA polymerase. The results from this experiment clearly indicate that reduced discrimination against fluorescein family dye-labeled nucleotides results from the E681K mutation rather than the G46D or F667Y mutations. In particular, the G46D F667Y double mutant DNA polymerase requires 25 times more Zowie-ddCTP, a fluorescein family dye-labeled nucleotide, than ddCTP to inhibit DNA synthesis by 50%, while the G46D F667Y E681K triple mutant DNA polymerase is inhibited equally by Zowie-ddCTP and ddCTP. Thus, the G46D or F667Y double mutant had no reduced discrimination against fluorescein family dye-labeled nucleotides. Therefore, the specification demonstrates that reduced discrimination against such dyes results from the E681K mutation, not the G46D or F667Y mutations.

In addition, the G46D mutation is described in the specification as attenuating the 5'-nuclease activity of the polymerase. *See* the specification at page 29, lines 11-19. This mutation is also extensively discussed in U.S. Patent No. 5,466,591 ("the '591 patent"), which is cited by the specification of the present application. *See* the specification at page 29, lines 13-14. The '591 patent also describes the G46D mutation as reducing the 5'-nuclease activity of the polymerase. The Declaration of David Gelfand further explains that mutations at position 46 do not affect the polymerase's ability to discriminate against fluorescein family dye-labeled nucleotides as position 46 lies in a completely separate structural domain from the critical motif responsible for this activity. *See* the Declaration of David Gelfand, paragraph 7. Furthermore, the Declaration of David Gelfand shows that the G46D mutation is not responsible for the reduced discrimination against fluorescein family dye-labeled nucleotides because the activities of the 19 E681x polymerase mutants are compared to a parent polymerase that is not mutated at position 681 but does contain the G46D mutation. Thus, Applicants respectfully submit that the F667Y and G46D mutations do not affect the ability of a recombinant thermostable DNA polymerase to discriminate against fluorescein family dye-labeled nucleotides.

As for the PTO's second objection, Applicants respectfully submit that the specification describes a sufficiently representative number of recombinant thermostable DNA polymerase species which are mutated at position four of the critical motif to an amino acid other than glutamate to convey to one skilled in the art that Applicants possessed the recombinant thermostable DNA polymerases at the time of filing. The PTO's attention is respectfully invited to the specification at, for example, page 11, lines 17-26. Here, the specification describes a number of specific mutations of position four of the critical motif, all of which have reduced discrimination against fluorescein family dye-labeled nucleotides. Furthermore, the Declaration of David Gelfand provides clear evidence that all recombinant thermostable DNA polymerases which are mutated at position four of the critical motif to an amino acid other than glutamate exhibit decreased discrimination against fluorescein family dye-labeled nucleotides. *See* the Declaration of David Gelfand, paragraphs 12-13.

Accordingly, Applicants respectfully submit that the specification sufficiently describes the invention to reasonably convey to one of skill in the art that Applicants were in

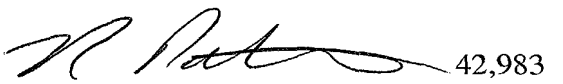
possession of the invention as presently claimed at the time of filing, as required by 35 U.S.C. § 112.

CONCLUSION

In view of the foregoing, Applicants believe that Claims 1-53 satisfy all criteria of patentability and are in proper condition for allowance. Early notification to that effect is therefore kindly solicited.

Respectfully submitted,

Date: January 3, 2003



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APPENDIX A

Marked-up Copy of Amended Claims

4. (Amended) The recombinant thermostable DNA polymerase of claim 2 wherein said polymerase is from a thermophilic species selected from the group consisting of Thermosipho africanus, Bacillus caldotenax, and Bacillus stearothermophilus.

5. (Amended) The recombinant thermostable DNA polymerase of claim 2 wherein said polymerase is from a Thermus species.

6. (Amended) The recombinant thermostable DNA polymerase of claim 5 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is any amino acid, and "Xaa" at position 6 is Ser or Ala; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu[; and].

[c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.]

7. (Amended) The recombinant thermostable DNA polymerase of claim [6] 5 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu[; and].

[c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.]

8. (Amended) The recombinant thermostable DNA polymerase of claim 7 [which is characterized in that] wherein said "Xaa" at position 4 is mutated to Lys.

9. (Amended) The recombinant thermostable DNA polymerase of claim 2 wherein said polymerase is from a Thermotoga species.

10. (Amended) The recombinant thermostable DNA polymerase of claim 9 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid and "Xaa" at position 7 is Val or Ile[.]; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu[; and].

[c said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in

comparison to the native form of said polymerase.]

16. (Amended) The nucleic acid sequence of claim 15 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or Gly, "Xaa" at position 4 is any amino acid, and "Xaa" at position 6 is Ser or Ala; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu[; and].

[c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.]

17. (Amended) The nucleic acid sequence of claim 15 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu[; and].

[c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.]

18. (Amended) The nucleic acid sequence of claim 17 [which is characterized in that] wherein said "Xaa" at position 4 is mutated to Lys.

20. (Amended) The nucleic acid sequence of claim 19 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid and "Xaa" at position 7 is Val or Ile[.]; and

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu[; and].

[c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.]

21. (Amended) A method of DNA sequencing which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile, and

ii) said polymerase has a [reduced] level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes [, and] which is reduced in comparison to the native form of said polymerase;

b) providing a dye-terminator labeled with a negatively charged fluorescent dye[,]; and

c) performing a dye-terminator sequencing reaction.

26. (Amended) The method of claim 25 wherein said amino acid sequence comprises[:] LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

29. (Amended) The method of claim 28 wherein said amino acid sequence comprises[:] LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

31. (Amended) A method of producing labeled DNA which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile[.], and

ii) said polymerase has a [reduced] level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

b) providing a nucleotide labeled with a fluorescein family dye[,]; and

c) performing a DNA synthesis reaction.

32. (Amended) A method of producing labeled primer extension products which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile[.],

ii) said polymerase has a [reduced] level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes[;] which is reduced in comparison to the native form of said polymerase,

iii) said polymerase also comprises the second amino acid sequence SQIXLR(V/I) (SEQ ID NO: 18) where "X" is any amino acid except E,

iv) said polymerase has a level of [reduced] discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

b) providing a ribonucleotide labeled with a fluorescein family dye[,]; and

c) performing a primer extension reaction.

33. (Amended) A kit for DNA sequencing which comprises:

a) a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile, and

ii) said polymerase has [reduced] a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes[,] which is reduced in comparison to the native form of said polymerase; and

b) a terminator labeled with negatively-charged fluorescent dye.

36. (Amended) The kit [Kit] of claim 35 wherein said "Xaa" at position 4 is Lys.

37. (Amended) The kit of claim 34 wherein said amino acid sequence comprises[:] LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

38. (Amended) The kit [Kit] of claim 37 wherein said "Xaa" at position 4 is Arg.

39. (Amended) A kit for DNA sequencing which comprises:

a) a mutant thermostable DNA polymerase characterized in that

i) in its native form said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 7 of this sequence is Val or Ile;

ii) said amino acid sequence is mutated, except that "Xaa" at position 4 is not mutated to Glu; and

iii) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.

42. (Amended) The kit [Kit] of claim 41 wherein said "Xaa" at position 4 is mutated to Lys.

44. (Amended) The kit [Kit] of claim 43 wherein said "Xaa" at position 4 is Arg.

45. (Amended) A kit for producing labeled DNA which comprises:

a) a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 7), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile,

ii) said polymerase has [reduced] a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes[,] which is reduced in comparison to the native form of said polymerase; and

b) a nucleotide labeled with a negatively-charged fluorescent dye.

46. (Amended) The kit of claim 45 wherein said amino acid sequence comprises[:] LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:14), whereby "Xaa" at position 4 is any amino acid except Glu.

47. (Amended) The kit [Kit] of claim 45 wherein said "Xaa" at position 4 is Lys.

48. (Amended) The kit of claim 45 wherein said amino acid sequence comprises[:] LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 15), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

49. (Amended) The kit [Kit] of claim 48 wherein said "Xaa" at position 4 is Arg.

50. (Amended) A kit for producing labeled primer extension products which comprises:

a) a thermostable DNA polymerase which is characterized in that

i) in its native form, the polymerase comprises the first amino acid sequence LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile;

ii) the polymerase has [reduced] a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase;

iii) the polymerase also comprises the second amino acid sequence SQIXLR(V/I) (SEQ ID No: 18) where "X" is any amino acid except E;

iv) the polymerase has [reduced] a level of discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase; and

b) a ribonucleotide labeled with a fluorescein family dye.

51. (Amended) The kit of claim 50 wherein said amino acid sequence comprises[:] LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

52. (Amended) The kit [Kit] of claim 51 wherein said "Xaa" at position 4 is Lys.

53. (Amended) The kit of claim 50 wherein said amino acid sequence comprises[:] LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

54. (Amended) The kit [Kit] of claim 53 wherein said "Xaa" at position 4 is Arg.